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Production of high quality *Ardisia* plants by stem tip cuttings

Mark S. Roh[a,*], Ae-Kyung Lee[b], Jeung-Keun Suh[b]

[a]*USDA, ARS, US National Arboretum, Floral and Nursery Plants Research Unit, B-010A, Rm 238, 10300 Baltimore Ave., Beltsville, MD 20705, USA*
[b]*Laboratory of Floriculture and Plant Physiology, School of Bio-Resources Science, Dankook University, Cheonan 330-714, Republic of Korea*

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Abstract

To produce commercially acceptable *Ardisia* plants, stem tip cuttings from mature plants were rooted and forced in greenhouses. Ten centimeter long cuttings were either treated with 200 ppm 1-naphthalene acetic acid (NAA) for 2 h, 2000 ppm indole-3-yl-butyric acid (IBA) for 10 s, or 0.5 and 1.0% IBA powder prior to sticking them in the rooting medium. Rooting percentage at 45 days exceeded 76% with 2000 ppm IBA treatment which was a significant increase over non-treated control. Rooted cuttings developed into three types of plants: those forming only vegetative shoots without flowers, those forming reproductive shoots with flowers, and those forming both vegetative and reproductive shoots. The ideal plant produced only vegetative shoots when rooted cuttings were transplanted into pots. About 50% rooted cuttings were forced to finish, producing 31 or 40% of high quality plants when rooted cuttings with vegetative shoots were grown in a greenhouse (GH) at temperatures higher than 21/19 °C (day/night) in 1995 or 21/18 °C GH in 1997, respectively. This method shortened the total production time to less than 2 years as compared to 4 years when starting from seeds.

Keywords: *Ardisia crenata*; Juvenility; Maturity; Cutting propagation; Forcing

Abbreviations: IBA, indole-3-yl-butyric acid; NAA, 1-naphthaleneacetic acid

* Corresponding author. Tel.: +1 301 504 5659; fax: +1 301 504 5096.
E-mail address: rohm@ba.ars.usda.gov (M.S. Roh).

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1. Introduction

The genus *Ardisia* consists of more than 200 species, generally grown in warm climates of subtropical and tropical areas (Bailey, 1925). *Ardisia japonica* BL., and *A. crenata* Sims. are the most widely grown species. *Ardisia crenata* has been mass produced by seeds, although selected germplasm with horticultural merit should be propagated vegetatively. *Ardisia* has been produced primarily as an indoor foliage plant in the past (Conover and Poole, 1989) and interest could increase when sold with bright red or white berries. Plants typically flower in June and red or white berries are produced around September (Lee, 1998; Lee et al., 2002). However, environmental factors, such as optimum temperature and photoperiod on flowering and berry development, have yet to be investigated. Berries remain attached 12 months or more in low light interior environments.

For many woody plants, a juvenile phase exists when plants are unable to flower. A long juvenile period had prevented extensive investigation of the controlling mechanism of flowering for *A. crenata*. The juvenile period of *A. crenata* can last 2–3 years (Roh, unpublished data) when seedlings are grown in a greenhouse maintained at 18.5/18.0 °C year-round (Lee and Roh, 2001). Only 25% of 2-year-old *A. crenata* seedlings flowered. When started from seeds, only a few shoots produced berries, thus producing plants with a few berries. Rooted stem tip cuttings from mature plants could be a useful production technique to bypass the juvenile period so that new plants could be induced to flower sooner. Cuttings obtained from the mature ivy, *Hedera helix*, retained the capacity to flower for many years even at the high temperatures favoring reversion to juvenile conditions (Wareing and Phillips, 1978). This research was initiated to study the effect of auxins on the rooting of *A. crenata* cuttings and to determine if commercially acceptable high quality plants can be produced for marketing in less than 2 years.

2. Materials and methods

2.1. Vegetative propagation (Experiment 1)

Ten centimeter long, recently matured uniform stem tip cuttings, were obtained from 3-year-old *A. crenata* plants grown in 4.8 L pots (L) in a greenhouse (GH) maintained at 21/16 °C, D/N, in March 1996. All stock plants flowered in the previous year. Cuttings under a vegetative stage were taken from the shoots that were formed above the canopy of shoots with berries that were formed in the previous years. Four to six leaves were attached in clusters at the tip of the stems, and all leaves were retained either during treatment or before sticking cuttings. Cuttings were trimmed to 7–8 cm long and either treated with 200 ppm 1-naphthaleneacetic acid (NAA) for 2 h, 2000 ppm indole-3-yl-butyric acid (IBA) for 10 s, or 0.5 and 1.0% IBA powder prior to sticking them in the rooting medium. Solutions of NAA and IBA were first dissolved in 5 ml of ethanol, and water was added to make the final concentrations. Thirty-five cuttings were used per replication, and three replications were used per treatment. Cuttings were stuck into a rooting medium composed of vermiculite:perlite:peat moss (1:1:1, by volume) in a completely randomized, block

design and propagated under a plastic tent as described (Lee, 1998). Temperature inside the tent maintained between 19 °C (minimum) and 30 °C (maximum).

2.2. Vegetative propagation (Experiment 2)

In April 1995, 10 cm long cuttings were collected from 4-year-old stock plants grown in a greenhouse maintained at 18.5/18.0 °C greenhouse. All stock plans flowered in previous years. Cuttings were treated with 0, 1000, 2000, or 4000 ppm of water-soluble potassium salt of IBA (IBA-K) solutions for 5 min and propagated under intermittent mist. Cuttings received 8 s of intermittent mist every 30 min for 24 h under natural irradiance and photoperiod in a greenhouse maintained at 23/21 °C. Cuttings were stuck into a rooting medium composed of Promix (Stamford, Conn, USA):vermiculite (1:1 by volume). Twelve cuttings were used per replication, and six replications were per treatment.

Data on rooting percentage, the number of roots (roots longer than 1 mm in length), lengths of the longest roots, and length of rooting zones on the base of the stem were recorded after 40 days (Experiment 1) and 45 days (Experiment 2). Rooting percentage data were arcsine transformed and analyzed. Data were subjected to a one-way analysis of variance (SAS Institute, 1999) and values obtained by Tukey's-test [honestly significant difference (hsd)] at the 5% significance level are presented to compare means.

2.3. Production of finished plants (Experiments 3 and 4)

On 18 June 1995, three-hundred 10 cm long terminal cuttings from shoots without berries were collected from 4-year-old stock plants, treated by dipping cuttings in 1500 ppm IBA-K solutions for 1 min, and propagated with intermittent mist at a 25/23 °C (D/N) GH (Experiment 3). On 9 October, 150 uniform rooted cuttings with vegetative shoots were transplanted, one per 0.78 L pot, filled with ProMix-BX.

One month after transplanting, one half of the plants was grown in a GH maintained at 15/13 °C (D/N) and the other half were grown at 21/19 °C (D/N) for temperature treatments (Experiment 3). On 6 January 1997, all plants were moved to a GH maintained at a minimum of 24/21 °C (D/N) and grown until the completion of experiment. Data were collected, and plants were visually assessed for ''quality'' as follows on October 1997. High quality plants as shown in the previous publication (refer to Fig. 1 in Lee et al., 2002) that can be accepted for commercial production belong to the group of A2, A3, or B4 described below.

A: Vegetative shoots at the upper part of the plant and reproductive shoots with berries beneath the vegetative shoots.

A1: Fewer than three shoots with berries—About three to six berries per cluster produced; more vegetative shoots than berry bearing shoots; no commercial value.

A2: Five to eight shoots with berries—Most shoots had five to eight berries per cluster; berry bearing shoots unevenly spread over the rim of the pot; some commercial value.

A3: More than eight shoots with berries—Most shoots had more than five to eight berries per cluster; berry bearing shoots evenly spread over the pot rim; high commercial value.

B: Berries produced from the shoots on the upper part of the plant.

B1: Fewer than two shoots with berries—Berries generally small, less than 2 mm in diameter; less than five berries per cluster; plants weak; no commercial quality.

B2: Three to five shoots with berries—Berries fully developed (3 mm in diameter); about five berries per cluster, but many fell off; plants appeared normal; the diameter (spread) of plant less than the size of the pot; low commercial quality.

B3: Six to eight shoots with berries—Berries fully developed (3 mm in diameter); five to eight berries per cluster; the diameter of plant slightly larger than the pot size; moderate commercial quality.

B4: More than nine shoots with berries—Berries fully developed; the diameter of plants 1.5 times greater than that of pot; high commercial quality.

The following data were collected from the finished plants. Plant height was measured from the surface of the growing medium to the origin of vegetative shoots (final height) and also to the origin of reproductive shoots (final height, when no vegetative shoots were formed). Plant width was measured at the widest leaf tip-to-tip dimension and also at 90° to this line, representing the narrowest dimension, and the average of the widest and narrowest dimensions were used to represent the plant width (size). The number of vegetative shoots without berries and reproductive shoots with berries was counted. Using an ABS Digimatic Caliper (Mitutoyo Co., Japan), the berry diameter was recorded for the two largest berries from each cluster. One cluster per stem and five stems per plant were measured.

Greenhouse temperature and plant type were included in the model of analysis, and these variables were analyzed using a one-way analysis of a variance (ANOVA) for a completely randomized design using the SAS program (SAS Institute, 1999). Values obtained by Tukey's-test [honestly significant difference (hsd)] at the 5% significance level are presented to compare means.

On 25 July 1997, 250 cuttings were treated with a 1000 ppm IBA-K solution as described above and propagated (Experiment 4). From rooted cuttings, 120 uniform plants that formed vegetative shoots without berries (type 1, Fig. 1) were selected for experiment. Between 16 December 1997 and 7 March 1998, 30 plants each were grown in a GH

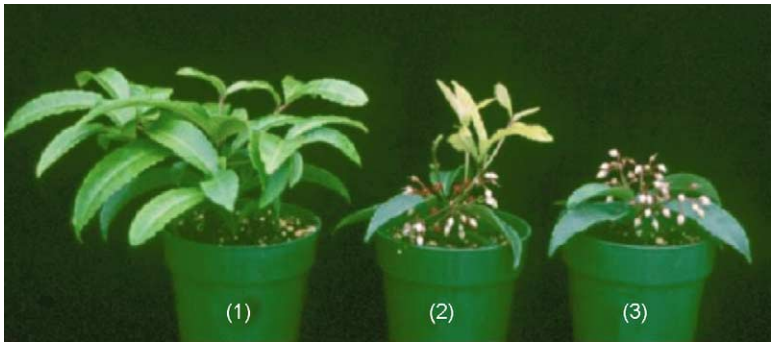

Fig. 1. Three types of *A. crenata* after transplanting of rooted cuttings. Type 1 plant produced only vegetative shoots, type 2 plant produced shoots with flowers and also with a few vegetative shoots, and type 3 plant produced shoots with flower buds only.

maintained at 15/12 °C, 18/15 °C, 21/18 °C, and 24/21 °C (D/N) for temperature treatment. After treatment, all plants were transferred to a GH maintained at a minimum of 21/18 °C until the completion of the experiment on 16 September 1998.

The dates of visible flower buds, anthesis and berry formation per group of plants were recorded when they reached the peak of the development stages described above. When the experiment ended, plants were divided into two groups, based on the type of new shoots with or without berries. Type A plants flowered and produced berries on both old shoots and new shoots, while type B for plants flowered and produced berries only on old shoots formed during rooting. The number of shoots with berries in type B plants was recorded. Berry-bearing and vegetative shoots were counted for all plants. The number of new vegetative shoots on type A plants that formed berries was recorded.

Finished plants were classified as unacceptable, marginal, acceptable, or superior based on the following criteria and plant types (Fig. 2). The number of berries per cluster was excluded during grading. Plants with the grade 4, acceptable, and grade 5, superior, were considered high quality that can be marketed commercially.

Grade 1: unacceptable—Fewer than four shoots with berries; only in type B1 plants.
Grade 2: unacceptable—Sometimes in type A1 plants with fewer than four shoots with berries; mainly in type B2 plants.
Grade 3: marginally acceptable—Less than six to seven shoots with berries; mainly type A2 plants, but some type B plants with more than 10 shoots with berries.

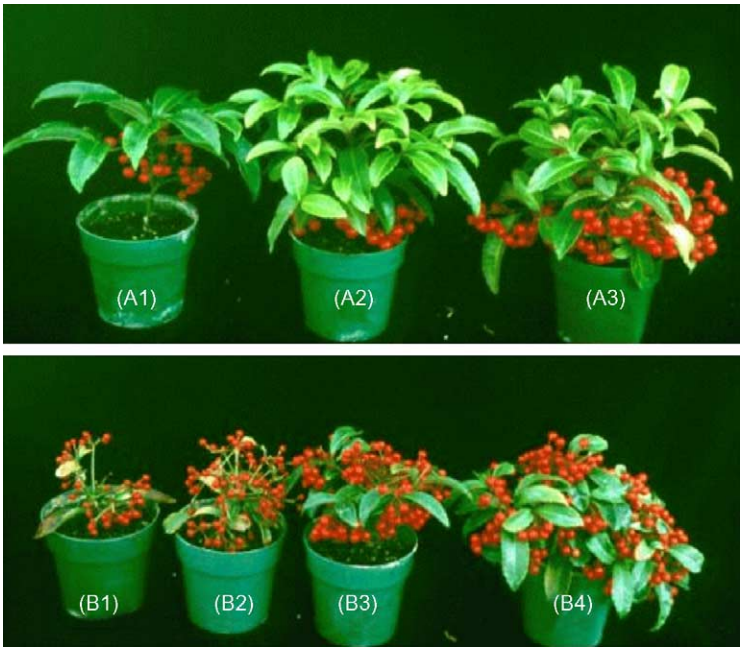

Fig. 2. Appearance of *A. crenata* produced from cuttings. Plants in group A formed berries beneath the vegetative shoots and plants in group B formed berries without the vegetative shoots.

Grade 4: acceptable—More than eight shoots with berries; no berries formed in the new shoots, only in type A3 and B3 plants.
Grade 5: superior—similar to Grade 4 plants, but berries formed in the new shoots; the highest quality; only in type B4 and A3 plants.

In both experiments, plants were fertilized with 0.8 g of 14N–6.2P–11.6K controlled-release fertilizer applied to the surface of the growing medium of individual pots, and plants were fertilized manually with a water soluble fertilizer (15N–7P–12.8K) once a month. Data were subjected to a one-way analysis of variance (SAS Institute, 1999) and values obtained by Tukey's-test [honestly significant difference (hsd)] at the 5% significance level are presented to compare means.

3. Results

3.1. Vegetative propagation

A. crenata cutting rooted successfully. Mean rooting percentage, the number of roots, root length, and a rooting zone increased significantly when cuttings were treated with 2000 ppm IBA as compared to those of the control (Experiment 1, Table 1). The number of roots and the length of the rooting zone was significantly increased by NAA and IBA 2000 ppm treatments. The number of roots, root length, and rooting zone were similar for cuttings treated with different types and concentration of IBA. When cuttings were treated with various concentrations of IBA-K solutions, rooting percentage varied from 64 (control) to 82% (1000 ppm IBA-K) (Experiment 2, data not presented). The number of roots per cutting varied between 0 and 26, but the means were similar regardless of the concentration of IBA-K solutions.

3.2. Types of growth

Three different plant types were observed about 1 month after transplanting the rooted cuttings (Fig. 1). Type 1 plants produced only vegetative shoots and were the largest. At transplanting, the number of vegetative shoots of type 1 plants ranged from 5 to 10 and the

Table 1
Effect of auxins on the rooting of *A. crenata* cuttings after 45 days (Experiment 1)

Treatment	Mean rooting percentage	Mean[a] no. of root	Mean[b] root length (cm)	Mean rooting zone (cm)
Control	64	4.5	3.7	3.0
NAA (200 ppm)	76	44.5	5.1	5.4
IBA (2000 ppm)	84	10.6	5.7	4.6
IBA 1% talc	84	9.2	4.6	3.8
IBA 0.5% talc	84	6.8	4.8	3.9
hsd at 5%	9	5.1	1.6	0.8

[a] Roots longer than 1 mm were counted.
[b] Length of the longest root.

length at transplanting ranged from 5 to 10 cm (data not presented). Type 2 plants produced shoots with reproductive shoots containing flower buds and also vegetative shoots, and type 3 plants produced shoots with only flower buds only and were the weakest in growth. When some extra plants of type 3 were pinched leaving four to six leaves that were formed before taking cuttings, they failed to survive.

3.3. Production of commercial quality potted plants

Only about 50% of the cuttings [150 rooted cuttings/300 (Experiment 3, Table 2) and 120 rooted cuttings/250 (Experiment 4)] were rooted well and produced type 1 plants only

Table 2
Growth of *A. crenata* from rooted cuttings as influenced by greenhouse (GH) forcing temperatures (Experiment 3)

GH temperature (°C)	Plant type[a]	No. of plant	Plant height (cm)[b]		Plant size (cm)[c]	Mean no. of shoots		Mean diameter of berry (mm)
			H1	H2		Vegetative shoots	Reproductive shoots	
15/13	A1	12	11	8	16.5	1.5	2.5	7.1
	A2	19	12	8	20.0	5.8	3.8	7.8
	A3	6	14	10	20.0	8.6	4.6	9.3
	B1	5	–[d]	7	7.5	1.8	2.4	6.8
	B2	13	–	8	10.0	2.6	3.2	8.7
	B3	16	–	10	13.5	2.4	4.3	9.3
	B4	0	–	–	–	–	–	–
21/19	A1	4	13	9	18.0	5.1	3.2	9.5
	A2	9	15	9	21.0	8.5	3.9	10.2
	A3	10	18	11	21.0	9.6	4.8	10.8
	B1	0	–	–	–	–	–	–
	B2	6	–	9	8.0	2.8	2.6	8.8
	B3	12	–	10	13.5	3.2	5.5	9.2
	B4	17	–	13	15.5	2.1	6.9	9.8
Significance								
Temperature			*	*	**	**	*	*
15/13 type A vs. B			–	ns[e]	**	**	*	*
21/19 type A vs. B			–	ns	**	**	*	**
hsd at 5%			3	3	4.7	1.2	2.2	1.0

[a] Type of plants with vegetative shoots at the upper part of the plant and reproductive shoots with berries beneath the vegetative shoots (A) and of berries produced from the shoots on the upper part of the plant (B). Refer to Section 2 for description of numeric number following A or B.

[b] Height from the surface of the growing medium to the stem where vegetative shoots formed (H1) or to the stem where reproductive shoots formed (H2).

[c] Average of plant width measured at the widest leaf tip-to-tip dimension and 90° to this line.

[d] No data collected since no plants were produced in these particular groups.

[e] Non-significant.

* Significant at $P < 0.05$.

** Significant at $P < 0.01$.

with vegetative shoots (Fig. 1) which were used for a pot plant production trial. Plants started to flower in late November at 21/19 °C and in early December 1995 at 15/13 °C and berries started to turn to red in September 1996, and color became intense by early December and by mid-November when plants were grown initially at 15/13 °C and 21/19 °C, respectively (Experiment 3). When rooted cuttings were grown at 15/13 °C, 12 plants died, whereas only four plants grown at 21/19 °C died. Losses may have been due to developing berries that may act as a strong sink competing for nutrients while too few leaves were present to supply nutrients to the berries. At the time of coloration of berries, plants were divided into three groups for the type A plants and four groups for the type B plants, depending on the position of the shoots with berries (Fig. 2).

More acceptable superior plants (B3 or B4 and also A3) were produced in a greenhouse maintained at 21/19 °C than at 15/13 °C (Experiment 3). The quality of plants grown initially at 21/19 °C as judged by the number of superior plants was higher than those grown at 15/13 °C. The average height of type A plants grown at 21/19 °C was taller than those that grown at 15/13 °C (Table 2). The plant size (spread) of type A plants and also type B plants were when grown in both greenhouses. For example, the size of type A3 plants grown at 15/13 °C and 21/19 °C was 20 and 21 cm, respectively. The number of vegetative shoots and reproductive shoots was higher in type A plants as compared to type B plants, but of berries was affected by temperature. The number of reproductive shoots was greater than 4.6 in type A3 and B3 plants when grown at 15/13 °C and greater than 4.8 when grown at 21/19 °C. The size of berries was larger when plants were grown at 21/19 °C than at 15/13 °C, and the size of berries in type A plants grown at 21/19 °C was greater than those grown at 15/13 °C. The largest berries, 10.8 mm in diameter, were obtained in type A3 plants grown at 21/19 °C. When rooted cuttings produced reproductive shoots with clusters of flower buds directly from the main stem (type 3 plants, Fig. 1), they grew poorly, and when these reproductive shoots were pinched, those plants failed to grow and produce any new shoots until the completion of experiment on 16 September 1998 (data not presented).

The number of type B plants decreased from 16 to 1 when the greenhouse temperature was increased from 15/12 °C to 24/21 °C (Experiment 4, Table 3). For example, the decrease in the number of type B1, B2, and B3 (B1 + B2 + B3) plants was inversely related

Table 3
Development of *A. crenata* from rooted cuttings as influenced by greenhouse (GH) forcing temperatures (Experiment 4)

GH temperature (°C)	Number of plants with berries from			Overall grade[b]	No. of dead plants
	Type B1 + B2 + B3	Type A2 + A3 + B4			
	Old shoots	Old shoots	New shoots[a]		
15/12	16	8	2	1.4	6
18/15	9	19	4	2.3	2
21/18	4	24	9	3.9	2
24/21	1	26	14	4.1	3
Total	30	77	29	–	13
hsd at 5%	–	–	–	0.7	–

[a] Both old and new berry-bearing shoots are included in counts of fruiting plants.
[b] Grade scale: (1) unacceptable; (2) unacceptable; (3) marginally acceptable; (4) acceptable; (5) superior.

to the increase in the temperature. On the contrary, when grown in greenhouses maintained at high temperatures, more plants produced reproductive shoots either from old shoots or from new shoots which formed after formation of old shoots with flowers and berries. When grown at 24/21 °C, 26 plants of type A2, A3, and B4 (Fig. 2) produced berries only from old shoots and 14 plants of these 26 plants produced berries from both old shoots and also new shoots as reported by Lee et al. (2002). The overall grade of plants grown in a GH at 21/18 and 24/21 °C were 3.9 and 4.1, respectively, which is significantly higher than the grade of plants grown at lower temperatures.

4. Discussion

Stem tip cuttings rooted in about 6 weeks. Rooting was increased by 2000 ppm IBA treatment and was higher than 82%. The percentage of rooting was comparable to the percentage of seeds that germinated (Lee, 1998). However, seeds required longer than 13 weeks at 25 °C to reach up to 80% germination, which is substantially longer than 45 days for cuttings to root. Among the three types of growth produced when rooted cuttings were transplanted (Fig. 1), only type 1 plants with only vegetative shoots were used in the subsequent production trial. Type 3 plants that produced shoots with flower buds did not grow and when these flowering shoots were pinched, they died. Since cuttings under a vegetative stage were uniform in the length and diameter and obtained only from the upper part of the stock plants, these variations could result from physiological nature of the rooted cuttings and not from a variation of stock plants or greenhouse environments.

If quality *A. crenata* plants can be produced by stem tip cuttings, the time to produce marketable plants can be reduced by about 6 weeks as compared to seed propagation when only the propagation period is considered. Information, however, on the percentage of high quality plants that can be produced when propagated from seeds lacking. The number of high quality plants was about 62% (36 plants in A2, A3, and B4 groups out of 58 at 21/19 °C) in the first year trial and 80% (24 plants out of 30 at 21/18 °C) to 86% (26 plants out of 30 at 24/21 °C) in the second year trials. Because only 31% (first year trial), or about 40% (second year trial) of cuttings taken for rooting developed into acceptable grades, cultural methods should be improved to increase the percentage of high quality plants. Based on 2 years of trial, the best temperature regime to promote flowering and berry production is to force them at 24/21 °C.

To increase the percentage of commercially acceptable plants as reported previously (Lee et al., 2002), measures should be taken to increase the percentage of plants which produce only vegetative shoots (type 1, Fig. 1). Could this be possible if cuttings are propagated at set temperatures higher than 25/23 °C, temperature regimes set during mist propagation period or growing rooted cuttings at temperatures higher than 24/21 °C. In the native habitat of *Ardisia*, maximum average temperature during June to September was higher than 29.6 °C (Lee, 1998). Irradiance or photoperiod could be another environmental factor to consider. Once the percentage of high quality plants is increased, standards to evaluate the quality of *A. crenata* could be further refined. The ability to flower in *A. crenata* cuttings was maintained when cuttings were obtained from mature plants, as for *H. helix* (Wareing and Phillips, 1978). Although many areas of research have been investigated to control the flowering of woody

plants (Meilan, 1997), a practice of accelerating flowering by rooting cuttings from adult plants for propagation had yet to be studied.

A total of 6 years would be needed if cuttings are taken from 4-year-old stock plants to produce quality plants in 2 years from taking cuttings. Production of growing stock plants outdoor to supply cuttings, and then force the rooted cuttings to produce quality berry-bearing plants should be developed. When 2-year-old plants were forced to flower after high temperature (35 °C) treatment for 30, 60, and 90 days, the quality of plants was poor since less than three shoots per plant were produced and four to eight berries per shoot is produced (Roh, unpublished data).

To increase the percentage of quality *A. crenata* plants as reported in this study, factors that control the formation of vegetative and reproductive shoots by rooted cuttings should be further investigated. Additional studies are required to increase the percentage of commercially acceptable plants, possibly by manipulating the flowering time of stock plants so that cuttings can be taken earlier than June and by growing plants at higher temperatures upon transplanting or longer than 80 days at temperatures tested in this study.

5. Conclusion

High quality *A. crenata* plants can be produced in less than 2 years from rooted cuttings. This is the first report of producing high quality *A. crenata* plants produced from rooted cuttings. Although the effect of rooting hormones on rooting varies, treating cuttings with 2000 ppm IBA improved rooting percentage and the number of roots formed. High quality plants when rooted cuttings with vegetative shoots were grown in a greenhouse (GH) at temperatures higher than 21/19 °C (day/night) in 1995 or 21/18 °C GH in 1997, respectively. This method shortened the total production time to <2 years when starting from cuttings obtained from mature stock plants as compared to 4 years when starting from seeds. To increase the percentage of plants that are considered high quality for commercial production, propagating and forcing at higher temperature could be considered. Further, A1 plants with no commercial value, which produces more vegetative shoots than berry bearing shoots, could be used to produce high quality plants for subsequent years or as source of further vegetative shoots for propagation.

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